REMARKS

In view of the above amendments and the following remarks, reconsideration of the outstanding office action is respectfully requested.

Rapid, reliable, and sensitive nucleic acid detection assays are extremely important in the field of molecular biology and genetics. Nucleic acid detection assays can be used in a wide variety of applications, including, but not limited to: (1) pathogen detection; (2) disease diagnostics; (3) genotyping; and (4) expression studies. The usefulness of a nucleic acid detection assay is often tied to its efficiency, reliability, sensitivity, and cost-effectiveness. These characteristics are easily evident in the area of genotyping, which relies heavily on a high-throughput format in order to yield meaningful results in a cost-effective manner.

Due in part to the significant public interest in genomics research, there is a need for increasingly reliable and economic genotyping assays. An ideal genotyping assay would have a number of features: (1) it would be easily automatable; (2) it would be quantitative (e.g., it can measure the relative concentrations of different alleles in a sample); (3) it would discriminate between all non-identical alleles; (4) it would not require expensive equipment or expensive reagents; and (5) it would not require knowledge of the exact nature of differences among alleles in order to discriminate them from one another.

Although there are a number of effective nucleic acid detection assays currently available, many of them are tedious, costly, and time-consuming. Further, a large number of these assays require multiple handling steps, which can adversely affect the reliability of the results due to contamination problems. Frequently, low concentrations of the target nucleic acid molecule of interest contribute to the inability to detect the target nucleic acid molecule in the sample. The development of the polymerase chain reaction ("PCR") as a method for amplifying nucleic acids in samples has revolutionized modern life sciences research, and has improved the ability to develop sensitive and reliable nucleic acid detection assays. The basic PCR method is described in detail in U.S. Patent Nos. 4,683,195, 4,683,202, and 4,800,159. Over the years, numerous PCR-based techniques have been developed for a variety of applications. These assays have greatly enhanced the ability to amplify nucleic acids and to obtain direct sequence information from as little as one copy of a target nucleic acid sequence.

PCR is typically performed by placing a sample nucleic acids mixture in a thermocycler and subjecting the samples to three distinct temperature cycles, commonly referred to in the art as the denaturing, annealing, and synthesizing stages. The sample mixture typically comprises the target nucleic acid molecule (often comprising a double-stranded DNA molecule), a mixture of deoxynucleoside triphosphates, a pair of primers, a heat stable DNA polymerase (e.g., *Taq* polymerase), and a buffer solution. The primers are specific to and define the nucleic acid region targeted for amplification. In the denaturation stage, the temperature is raised to a temperature sufficient to separate the two strands of the DNA sample, resulting in single-stranded DNA templates for amplification. In the next stage of the cycle (i.e., the annealing stage), the temperature is lowered to allow for the generation of primed templates, during which stage the primers anneal to the single-stranded target templates. In the third stage of the cycle (i.e., the synthesizing stage), the temperature is raised to allow for binding of the DNA polymerase and for synthesis of the target nucleic acid. The cycle of strand separation, annealing of the primers, and synthesis of the target nucleic acid is repeated for about 20 to 60 cycles. The resulting nucleic acid molecule copies made in a given cycle serve as templates for the succeeding cycle. The number of target nucleic acid molecule copies increases approximately two-fold in each cycle. Although PCR is a powerful tool, recovery of amplification products may require the performance of tedious purification procedures, such as organic extraction, gel electrophoresis, centrifugation, and/or column purification.

Modified microtiter wells are well known in the art as a means for capturing PCR products, commonly referred to in the art as "amplicons," on a solid support (also referred to herein as a "solid substrate") prior to hybridization. For example, in one reported method, 5'-phosphorylated DNA primers are bound to secondary amines on microtiter well surfaces using standard carbodiimide condensation. However, many of the methods involving the capture of amplicons on solid supports such as microtiter wells still require the amplicons to be transferred from one well to another during the process, thereby causing problems due to contamination.

It is known in the art that combining the PCR amplification and immobilization stages into a single step is useful in decreasing the risk of contamination and in improving the efficiency of the amplification process. For example, various covalent chemical attachment methods have been analyzed for immobilizing one of the PCR primers in a pair onto controlled pore glass ("CPG") and/or polymer supports. In such methods, bead-

bound primers are used to amplify and covalently immobilize one or more DNA amplicons simultaneously. This method results in the ability to more easily manipulate sequences and eliminates the need to conduct extensive PCR product purification steps. It further allows for the use of the PCR products in subsequent applications; i.e., beyond the confines of a test tube, glass slide, or microtiter plate.

Solid-phase PCR ("SP-PCR") is a variation of the standard PCR method. SP-PCR can be used in a variety of applications and can overcome some of the problems associated with standard PCR protocols. There are several types of SP-PCR protocols. Some SP-PCR methods involve attaching the PCR products to a solid substrate after PCR amplification. Other protocols involve attaching a primer to the solid substrate and then conducting PCR amplification, resulting in bound amplicons. Assays that involve post-PCR immobilization of the amplicons are available for numerous applications, including, without limitation, the following: the detection of single or multiple nucleotide polymorphisms; the identification of bacterial agents; genetic phylogeny analysis and hybridization assays for gene detection; *in vitro* transcription; and the development of cDNA microarrays for analysis of gene expression. Using these protocols, very small amounts of target nucleic acid molecules can be amplified and, therefore, detected using detection labels. For example, immobilization of the amplicons onto solid substrates can be combined with colorimetric or fluorescent signal generating labels, thereby facilitating the identification and quantification of the target nucleic acid molecules in a sample.

A major difference between standard PCR and SP-PCR procedures, is that in standard PCR protocols the oligonucleotide primers bind to template or target nucleic acid molecules in solution, while in SP-PCR protocols template or target nucleic acid molecules are hybridized to immobilized primers.

Microplate-based solid-phase extension products are usually detected by enzymatic assays. For some applications, however, it would be preferable to employ direct detection of fluorescent products, which would allow quantitative estimation of yield over a wide dynamic range, as well as having the advantages of simplicity, flexibility, and cost. Thus, it would facilitate the use of microplate-based SP-PCR in high-throughput, automated applications. However, SP-PCR yields have not been sufficient for direct fluorescence detection with standard plate readers, requiring ~100 femtomoles (fmol) of product per microplate well for reliable quantification.

Despite SP-PCR's potential, current SP-PCR protocols have serious practical limitations. Previous studies have demonstrated that steric hindrance inhibits the hybridization of DNA in solution to immobilized oligonucleotides. Steric hindrance can also affect solid-phase polymerization by impeding the attachment of *Taq* polymerase to tethered oligonucleotides that directly abut the supporting surface. It has been demonstrated that SP-PCR efficiency is enhanced when a polydeoxythymidine ("(dT)") spacer is included at the 5' end of the solid-phase primer. Solid-phase oligonucleotides containing 5' $(dT)_n$ spacers are desirable, because they are inexpensive and easy to synthesize. However, high background signals are often observed when using these primers to amplify AT-rich plant DNA templates.

The present invention is directed to overcoming the deficiencies in the prior art.

Claims 20 and 24-27 have been canceled. Claims 1, 10, 12, 18, and 21 have been amended. Claim 1 has been amended to recite that the "linking agent is generated using a 5'-amino modifier coupled to a polyethylene glycol spacer." Support for this amendment to claim 1 is found in the specification at page 16, lines 19-27, and page 18, lines 30-31. Claim 18 has been amended to recite that the "5'-amino modifier is a 5'-Amino Modifier C6 spacer." Support for this amendment to claim 18 is found in the specification at page 16, line 25, and page 17, lines 14-15. Applicants respectfully submit that no new matter has been added by the above amendments to the claims.

The rejection of claims 10 and 12 under 35 U.S.C. § 112 (2nd para.) for indefiniteness is respectfully traversed in view of the above amendments.

The rejection of claims 1-4, 9-17, 28, 29, 40, and 41 under 35 U.S.C. § 102(b) as anticipated by U.S. Patent No. 6,060,288 to Adams et al. ("Adams") is respectfully traversed in view of the above amendments and the following remarks. Adams teaches a method of amplifying and detecting target nucleic acids using primers attached to a solid support. As discussed above, claim 1 has been amended to recite that the "linking agent is generated using a 5'-amino modifier coupled to a polyethylene glycol spacer." Adams does not teach a method whereby the primer is coupled to the solid support by a linking agent, where the linking agent is generated using a 5'-amino modifier coupled to a polyethylene

glycol spacer. Further, the USPTO has acknowledged that Adams does not teach a method involving a linking agent containing a polyethylene glycol spacer (see Office Action at pages 10-11). Applicants respectfully submit that the amendments to claim 1 are sufficient to overcome this rejection. For these reasons, the rejection of claims 1-4, 9-17, 28, 29, 40, and 41 under 35 U.S.C. § 102(b) is improper and should be withdrawn.

The rejection of claims 1-4, 7, 9-11, 14, 17, 28, 29, 40, and 41 under 35 U.S.C. § 102(b) as anticipated by U.S. Patent No. 5,747,251 to Carson et al. ("Carson") is respectfully traversed in view of the above amendments and the following remarks. Carson describes a method of determining the presence and concentration of a target nucleic acid in a sample. Amplification of the target nucleic acid is achieved using primers that are anchored to a solid support. However, the method of Carson does not involve primers coupled to the solid support by a linking agent, where the linking agent is generated using a 5'-amino modifier coupled to a polyethylene glycol spacer. The USPTO has acknowledged that Carson does not teach a method involving a linking agent containing a polyethylene glycol spacer (see Office Action at page 12). For the above reasons, applicants respectfully submit that the rejection of claims 1-4, 7, 9-11, 14, 17, 28, 29, 40, and 41 under 35 U.S.C. § 102(b) is improper and should be withdrawn.

The rejection of claim 5 under 35 U.S.C. § 103(a) for obviousness over Adams in view of U.S. Patent No. 5,475,098 to Hall et al. ("Hall") is respectfully traversed in view of the above amendments and the following remarks. Hall is cited as teaching that *Escherichia coli* nucleic acids could be detected from biological samples using polymerase chain reaction ("PCR") and probes specific to the *E. coli* nucleic acids. Hall does not teach or suggest detection of target nucleic acid molecules using primers coupled to a solid support by a linking agent, where the linking agent is generated using a 5'-amino modifier coupled to a polyethylene glycol spacer. Thus, because Hall does not overcome the deficiencies of Adams (as described above), applicants respectfully submit that this rejection is improper and should be withdrawn.

The rejection of claim 5 under 35 U.S.C. § 103(a) for obviousness over Carson in view of U.S. Patent No. 5,652,106 to Pilkaytis et al. ("Pilkaytis") is respectfully traversed in view of the above amendments and the following remarks. Pilkaytis is cited as teaching that *Mycobacterium tuberculosis* nucleic acids could be detected from biological samples using PCR and probes specific to the *M. tuberculosis* nucleic acids. Pilkaytis does not teach or suggest a method of detecting target nucleic acid molecules involving primers coupled to a solid support by a linking agent, where the linking agent is generated using a 5'-amino modifier coupled to a polyethylene glycol spacer. Thus, because Pilkaytis does not overcome the deficiencies of Carson (as described above), applicants respectfully submit that this rejection is improper and should be withdrawn.

The rejection of claim 6 under 35 U.S.C. § 103(a) for obviousness over Adams in view of U.S. Patent No. 5,489,513 to Springer et al. ("Springer") is respectfully traversed in view of the above amendments and the following remarks. Springer is cited as teaching that *Candida albicans* nucleic acids could be detected from biological samples using PCR and probes specific to the *C. albicans* nucleic acids. Springer does not teach or suggest the use of primers coupled to a solid support by a linking agent, where the linking agent is generated using a 5'-amino modifier coupled to a polyethylene glycol spacer. Thus, because Springer does not overcome the deficiencies of Adams (as described above), applicants respectfully submit that this rejection is improper and should be withdrawn.

The rejection of claim 6 under 35 U.S.C. § 103(a) for obviousness over Carson in view of Springer is respectfully traversed in view of the above amendments and the following remarks. The deficiencies of Carson and Springer are described above. In view of the amendments to claim 1 (from which claim 6 depends), applicants submit that the rejection of claim 6 under 35 U.S.C. § 103(a) is improper and should be withdrawn.

The rejection of claim 7 under 35 U.S.C. § 103(a) for obviousness over Adams in view of U.S. Patent No. 5,599,662 to Respess ("Respess") is respectfully traversed in view of the above amendments and the following remarks. Respess is cited as teaching the detection of HIV nucleic acids in biological samples using PCR and probes specific for the amplified HIV nucleic acids. Nowhere does Respess teach or suggest a method of detecting target nucleic acid molecules involving primers coupled to a solid support by a linking agent,

where the linking agent is generated using a 5'-amino modifier coupled to a polyethylene glycol spacer. Thus, because Respess does not overcome the deficiencies of Adams (as described above), applicants respectfully submit that this rejection is improper and should be withdrawn.

The rejection of claim 8 under 35 U.S.C. § 103(a) for obviousness over Adams in view of U.S. Patent No. 5,792,609 to Wataya et al. ("Wataya") is respectfully traversed in view of the above amendments and the following remarks. Wataya is cited as teaching that *Plasmodium falciparum* nucleic acids could be detected from biological samples using PCR and probes specific to the amplified *P. falciparum* nucleic acids. Wataya does not teach or suggest the use of primers coupled to a solid support by a linking agent, where the linking agent is generated using a 5'-amino modifier coupled to a polyethylene glycol spacer. Thus, because Wataya does not overcome the deficiencies of Adams (as described above), applicants respectfully submit that this rejection is improper and should be withdrawn.

The rejection of claim 8 under 35 U.S.C. § 103(a) for obviousness over Carson in view of Wataya is respectfully traversed in view of the above amendments and the following remarks. The deficiencies of Carson and Wataya are described above. In view of the amendments to claim 1 (from which claim 8 depends), applicants submit that the rejection of claim 8 under 35 U.S.C. § 103(a) is improper and should be withdrawn.

The rejection of claim 12 under 35 U.S.C. § 103(a) for obviousness over Carson in view of U.S. Patent No. 5,633,136 to Croce et al. ("Croce") is respectfully traversed in view of the above amendments and the following remarks. Croce discloses the use of nucleic acid probes for detecting human leukemias involving breakpoints on chromosome 11 in the ALL-1 locus. Nowhere does Croce teach or suggest the use of primers coupled to a solid support by a linking agent, where the linking agent is generated using a 5'-amino modifier coupled to a polyethylene glycol spacer. Thus, because Croce does not overcome the deficiencies of Carson (as described above), applicants respectfully submit that this rejection is improper and should be withdrawn.

The rejection of claim 13 under 35 U.S.C. § 103(a) for obviousness over Carson in view of U.S. Patent No. 5,298,392 to Atlas et al. ("Atlas") is respectfully traversed in view of the above amendments and the following remarks. Atlas is cited as teaching the detection of nucleic acids of potentially pathogenic organisms in a biological sample using PCR and probes specific for the amplified nucleic acids. The method of Atlas does not involve detecting target nucleic acid molecules using primers coupled to a solid support by a linking agent, where the linking agent is generated using a 5'-amino modifier coupled to a polyethylene glycol spacer. Thus, because Atlas does not overcome the deficiencies of Carson (as described above), applicants respectfully submit that this rejection is improper and should be withdrawn.

The rejection of claim 20 under 35 U.S.C. § 103(a) for obviousness over Adams in view of U.S. Patent No. 6,566,069 to Virtanen ("Virtanen") is respectfully traversed in view of the cancellation of claim 20.

The rejection of claim 20 under 35 U.S.C. § 103(a) for obviousness over Carson in view of Virtanen is respectfully traversed in view of the cancellation of claim 20.

The rejection of claims 20 and 21 under 35 U.S.C. § 103(a) for obviousness over Adams in view of U.S. Patent No. 6,429,300 to Kurz et al. ("Kurz") is respectfully traversed in view of the above amendments and the following remarks. Kurz is cited as teaching the use of a linking agent containing a polyethylene glycol spacer to attach oligos to a second substance. However, Kurz does not teach or suggest that the linking agent is generated using a 5'-amino modifier coupled to the polyethylene glycol spacer. With respect to claim 20, this rejection is rendered moot in view of the cancellation of claim 20. Regarding claim 21, because Kurz does not overcome the deficiencies of Adams (as described above), applicants respectfully submit that this rejection is improper and should be withdrawn.

The rejection of claims 20 and 21 under 35 U.S.C. § 103(a) for obviousness over Carson in view of U.S. Patent No. 6,582,918 to Janjic et al. ("Janjic") is respectfully traversed in view of the above amendments and the following remarks. Janjic is cited as teaching the use of a linking agent containing a polyethylene glycol spacer to attach oligos to

a substrate. Janjic does not, however, teach or suggest that the linking agent is generated using a 5'-amino modifier coupled to the polyethylene glycol spacer. With respect to claim 20, this rejection is rendered moot in view of the cancellation of claim 20. Regarding claim 21, because Janjic does not overcome the deficiencies of Carson (as described above), applicants respectfully submit that this rejection is improper and should be withdrawn.

The rejection of claims 28 and 37 under 35 U.S.C. § 103(a) for obviousness over Adams in view of U.S. Patent No. 6,022,700 to Monks et al. ("Monks") is respectfully traversed in view of the above amendments and the following remarks. Monks is cited as teaching the use of solid substrates containing microwells in biological assays using direct fluorescence detection. Monks does not teach or suggest the use of primers coupled to a solid support by a linking agent, where the linking agent is generated using a 5'-amino modifier coupled to a polyethylene glycol spacer. Thus, because Monks does not overcome the deficiencies of Adams (as described above), applicants respectfully submit that this rejection is improper and should be withdrawn.

The rejection of claim 37 under 35 U.S.C. § 103(a) for obviousness over Carson in view of Monks is respectfully traversed in view of the above amendments and the following remarks. The deficiencies of Carson and Monks are described above. In view of the amendments to claim 1 (from which claim 37 depends), applicants submit that the rejection of claim 37 under 35 U.S.C. § 103(a) is improper and should be withdrawn.

The rejection of claims 38 and 39 under 35 U.S.C. § 103(a) for obviousness over Adams in view of U.S. Patent No. 5,728,526 to George, Jr. et al. ("George") is respectfully traversed in view of the above amendments and the following remarks. George is cited as teaching the use of an extension mixture containing dATP, dCTP, dTTP, dGTP, dITP, and dUTP for use in extending nucleic acid strands. George does not teach or suggest a method of detecting target nucleic acid molecules involving primers coupled to a solid support by a linking agent, where the linking agent is generated using a 5'-amino modifier

coupled to a polyethylene glycol spacer. Because George does not overcome the deficiencies of Adams (as described above), applicants respectfully submit that this rejection is improper and should be withdrawn.

The rejection of claims 38 and 39 under 35 U.S.C. § 103(a) for obviousness over Carson in view of George is respectfully traversed in view of the above amendments and the following remarks. The deficiencies of Carson and George are described above. In view of the amendments to claim 1 (from which claims 38 and 39 depend), applicants submit that the rejection of claims 38 and 39 under 35 U.S.C. § 103(a) is improper and should be withdrawn.

The rejection of claim 42 under 35 U.S.C. § 103(a) for obviousness over Adams in view of U.S. Patent No. 6,312,929 to McMillan ("McMillan") is respectfully traversed in view of the above amendments and the following remarks. McMillan is cited as teaching the use of fluorescein as a fluorescent label in nucleic acid labeling methods. McMillan does not, however, teach or suggest a method of detecting target nucleic acid molecules involving primers coupled to a solid support by a linking agent, where the linking agent is generated using a 5'-amino modifier coupled to a polyethylene glycol spacer. Because McMillan does not overcome the deficiencies of Adams (as described above), applicants respectfully submit that this rejection is improper and should be withdrawn.

The rejection of claim 42 under 35 U.S.C. § 103(a) for obviousness over Carson in view of McMillan is respectfully traversed in view of the above amendments and the following remarks. The deficiencies of Carson and McMillan are described above. In view of the amendments to claim 1 (from which claim 42 depends), applicants submit that the rejection of claim 42 under 35 U.S.C. § 103(a) is improper and should be withdrawn.

The objection to claims 18, 19, 22-27, and 30-36 as depending from a rejected independent base claim is respectfully traversed in view of the above amendments.

In view of all of the foregoing, applicants submit that this case is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

Date: June 1, 2004

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